

Fig.

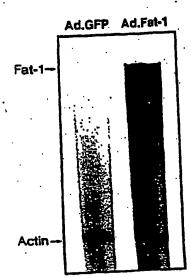
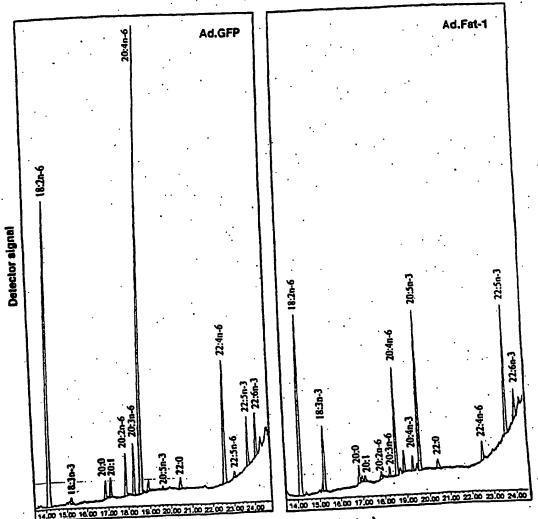


Fig. 2



Column retention time (minutes)

Fig. 3

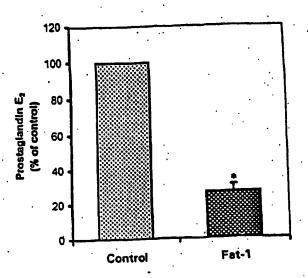


Fig. 4

	•	
Mol % of total Fatty acids	Control	Fat-1
n-6 Polyunsaturate	S .	
18:2n-6	14.2°	9.2 ^b
20:2n-6	1.2°.	0.3 ⁶
20:3n-6	1.6ª	0.4 ^b
20:4n-6	15.2°	4.1 ^b
22:4n-6	4.4°	1.0 ^b
	0.2ª	O.0 ^b
22:5n-6	36.8°	15.0 ^b
Total	•	
n-3 Polyunsaturāte	98 - ab	3.6ª
18:3n-3	0.2 ^b	0.6ª
20:4n-3	0.0 ^b	
20:5n-3	0.1 ^b	6.1 ^a
22:5n-3	1.2 ^b	5.8ª
22:6n-3	1.0 ⁸	1.3ª
Total	2.5 ^b	17.4°
n-6/n-3 Ratio	14.7°	0.9 ^b

Values are means of four measurements. Values for each fatty acid with the same letter do not differ significantly (P<0.01) between control and fat-1.

Fig. 5

into cultured cells	→ Assessment of expression → of the transgene	examination of activity of the n-3 desaturase	effect on lipid profiles		
Ģ	8 .	\$.	8		
Construction of viral expression vector coll culture	•mRNA (Northern) analysis •Protein (Western) analysis	 Enzymatic assay with radiolabeled fatty acids GC analysis 	Lipid extraction & TLC Fatty acid methylation FA analysis by GS-MS		

idlet for >	Gene transfer > (Viral delivery)	Organ harvest at day 2, 4, 10, 30 & 60 after gene transfer	÷	Biochemical analysis		
0 days rmal diet, gh n-6 FA diet	8 Ad.GFP (8 rats x 5) Ad.Fat-1 (8 rats x 5)	(8 rats/per time point)	F	8 In transcripts (mRNA) Setty acid composition Setty and contents		
oh n-3 FA diet		endpoint reference)		•		

Vector >
onstruction

AG + Fat-1

:loning

 Mice breeding → Tissue harvest and feeding

Normal diet or high n-6 FA diet Biochemical analyses &

Gene transcripts (mRNA Fatty acid composition Eleosanoid contents

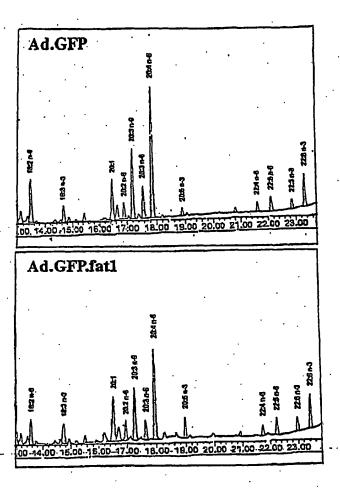


Fig. 9

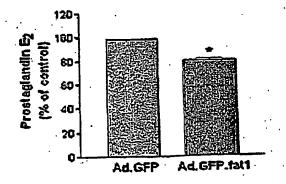
PUFA composition of total cellular lipids from the control rat cortical and the transgenic cells expressing a C. elegans fat-1 cDNA

Mol % of total fatty acids	Control	fat-1	
n-6 Polyunsaturates			
18:2n-6	1.78	0.87	
20:4n-6	7.21	4.23	
22:4n-6	1.57	0.72	•
22:5n-6	1.68	0.72	
Total	12.26	6.53	
n-3 Polyunsaturates			
18:3n-3	0.34	0.86	·
20:5n-3	0.21	0.87	
22:5n-3	0.29	0.81	
22:6n-3	1.27	1.93	
Total	2.11	4.48	
n-6/n-3 Ratio	6.44	1.67	

Values are means of four measurements. (p<0.01) between control and fat-1.

Fig. 10





MTT Assay

Fig.12

Ad.GFP.



Ad.GFP.Fat-1



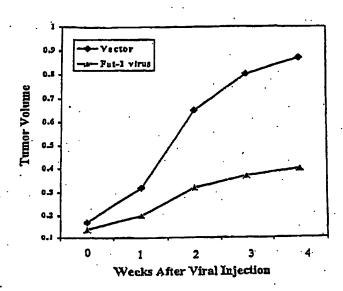


Fig . 14

PUFA composition of total cellular lipids from the control MCF-7 and the transgenic cells expressing a C. elegans fat-1 cDNA

O. Stopman	·						
Mol % of total fatty acids	Control Fat-1						
n-6 Polyunsaturates		-					
18:2n-6	3.13°	1.51 ^b					
20:2n-6	0.23°	0.22°					
20:3n-6	0.34 ³	0.16 ^b					
20:4n-6	6.30°	2.26 ^b					
22:4n-6	0.53	0.33 ^b					
22:5n-6	0.27 ⁹	0.11 ^b					
Total	. 10.80°	4.59 ^b					
n-3 Polyunsaturates	·.						
18:3n-3	0.0 ^b	1.00°					
20:4n-3	0.0 ^b	0.10					
20:5n-3	0.0 ^b	2.87					
22:5n-3	0.33 ^b	1.47					
22:6n-3	0.60 ^a	0.73 ^a 6.17 ^b					
Total	0.93 ^b	6.17					
n-6/n-3 Ratio	11.61°	0.74 ^b					

Values are means of four measurements. Values for each fatty acid with the same letter do not differ significantly (p<0.01) between control and fat-1.

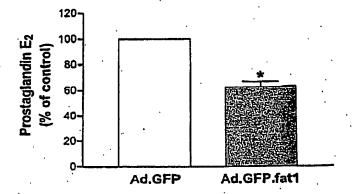


Fig. 16

CAAGTITGAG GT ATG GIC GCT CAT TOC TOA SAA GGG TTA TOC GCC ACG GCT CCG GTC Met Val Ala Ris Ser Ser Glu Gly Leu Ser Ala Thr Ala Pro Val 102 ACC GGC GGA GAT GTT CTG GTT GAT GCT CGT GCA TCT CTT GAA GAA Thr Gly Gly Asp Val Leu Val Asp Ala Arg Ala Ser Leu Glu Glu . AAG GAG GCT CCA CGT GAT GTG AAT GCA AAC ACT AAA CAG GCC ACC Lys Glu Ala Pro Arg Asp Val Asn Ala Asn Thr Lys Gln Ala Thr 40 35 ACT GAA GAG CCA CGC ATC CAA TTA CCA ACT GTG GAT GCT TTC CGT 192 Thr Glu Glu Pro Arg Ile Gln Leu Pro Thr Val Asp Ala Phe Arg 55 . CGT GCA ATT CCA GCA CAC TGT TTC GAA AGA GAT CTC GTT AAA TCA 237 Arg Als Ile Pro Als His Cys Phe Glu Arg Asp Leu Val Lys Ser 70. ATC AGA TAT TTG GTG CAA GAC TTT GCG GCA CTC ACA ATT CTC TAC Ile Arg Tyr Leu Val Gln Asp Phe Ala Ala Leu Thr Ile Leu Tyr TTT GCT CTT CCA GCT TTT GAG TAC TTT GGA TTG TTT GGT TAC TTG Phe Ala Leu Pro Ala Phe Glu Tyr Phe Gly Leu Phe Gly Tyr Leu 100 GTT TGG AAC ATT TTT ATG GGA GTT TTT GGA TTC GCG TTG TTC GTC Val Trp Asn Ile Phe Met Gly Val Phe Gly Phe Ala Leu Phe Val 110. GTT GGA CAC GAT TGT CTT CAT GGA TCA TTC TCT GAT AAT CAG AAT Val Gly His Asp Cys Leu His Gly Ser Phe Ser Asp Asn Gln Asn CTC AAT GAT TTC ATT GGA CAT ATC GCC TTC TCA CCA CTC. TTC TCT Leu Asn Asp Phe Ile Gly His Ile Ala Phe Ser Pro Leu Pho Ser 140 CCA TAC TTC CCA TGG CAG AAA AGT CAC AAG CTT CAC CAT GCT TTC 507 Pro Tyr Phe Pro Trp Gln Lys-Ser-His-Lys-Leu His His Ala Phe 155 ACC AAC CAC ATT GAC AAA GAT CAT GGA CAC GTG TGG ATT CAG GAT Thr Asn His Ile Asp Lys Asp Eis Gly His Val Trp Ile Gln Asp 175 170 AAG GAT. IGG GAA GCA ATG CCA TCA TGG AAA AGA TGG, TTC AAT CCA Lys Asp Trp Glu Ala Het Pro Ser Trp Lys Arg Trp Phe Asn Pro 190 185 ATT CCA TTC TCT GGA TGG CTT AAA TGG TTC CCA GTG TAC ACT TTA . 642 Ile Pro Phe Ser Gly Trp Leu Lys Trp Phe Pro Val Tyr Thr Leu 200 TTC GGT TTC TGT GAT GGA TCT CAC TTC TGG CCA TAC TCT TCA CTT

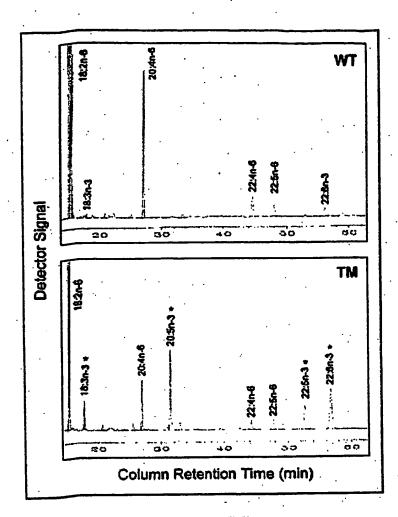
Fig. 17A

Phe	Gly	Phe	Cys	Asp 215	61 y	Ser	His	Phe	Trp 220	Pro	Tyr _.	Ser	Ser	Leu 225	
TTT Phe	GTT Val	CGT	AAC Asn	TCT Ser 230	GAC Asp	CGT Arg	GTT Val	CAA Gln	TGT Cys 235	GTA Val	ATC Ile	TCT Ser	CTA	ATC 11e 240	732
TGT Cys	TGC Cys	TGT Cys	GTG Val	TGT Cys 245	GCA Ala	TAT Tyr	ATT Ile	GCT Alb	CTA Leu 250	ACA Thr	ATT	GCT Ala	GI y	Ser 255	רדר
TAT	TCC Ser	AAT Asn	TGG Trp	TTC Phe 260	TGG Trp	TAC Tyr	TAT Tyr	TGG	GTT Val 265	Pro	CTI	TCT Ser	TTC Phe	TTC Phe 270	822
GGA Gly	Ten	ATG Met	CTC	GTC Val 275	ATT	GTT Val	ACC Thr	TAT Tyr	TTG Leu 280	GII	CAT His	GTC Val	GA?	GAT Asp 285	8 67
GTC Val	GCT Ala	GAG Glu	GTG Val	TAC Tyr 290	GAG Glu	GCT Ala	GAT Asp	GAA Glu	TGG Trp 295	Ser	·TTC	GT(CG!	GGA G Gly 300	912
CAA Gln	ACC Thr	CAA Gln	ACC Thr	ATC Ile 305	GAT Asp	CGT Arg	TAC Tyr	TAI	GGA G1y 310	Lei	GGI	A TTV	G GA	C ACA p Thr 315	
ACG Thr	ATG Met	CAC His	CAT His	ATC Ile 320	Thr	GAC Asp	GGA Gly	CAC His	: GT1 : Val 32:	(VT	CA:	T CA	c TT s Ph	C TTC e Phe 330	•
AAC Asn	AAA Lys	ATC Ile	CCA Pro	CAT His 335	Tyr	CAT	CTC	TA C	GAI Glu 340	3 AL	A AC B Th	C GA r Gl	A GG U GI	of GTC y Val 345	•
AAA Lys	AAG Lys	GTC Val	TTG Leu	GAG G1u 350	Pro	TTG Lev	TC(Sez	GAG Asi	P Th	E GT	A TA	r GI	y T	Yr Lys 360	• .
Ser	Gl'n	Val	Aşr	369	Asp	Ph€	Pho	e Al	a AI 37	g Pn 0.	ie m	20 14		TC AAG he Asi 37.	5
TAC	AAG Lys	CTC	GAC Asp	TAT TYI 380	Let	GTT Val	r CA L Hi	C AA s Ly	G AC s Th	E W	C G	SA A' ly I	TC A le M	TG CA let Gl 39	0 : .
TTC Phe	CGA Arg	ACA	AC:	CTC Lev 39	ı Glı	GAC	S AA u Ly	e <i>PT</i>	A AF	/S A.	CC A la L	ag t Ys	AA	·. 	1221
	ATA					•									1271
CCGGTTTTGC TCTAATTGCA ATTTTCTTT GITCTATAA							1321								
					•	AAAT	AAAC	TT (TAC	TTTT	CA (TGC	JT TG	AA	1371
TG	ATA:	AGC	CAT	AACT	CTT								•		1371

Fig. 17B

ODCIMIZED TALLY ONLY

caa gtt tga ggt ATG gtc gct cat too AGC gaa ggg CtG too goo acg gct cog gtc acc ggc ggC gat gtG ctg gtG gat gcC cgt gca tct ctG gaG gaG aag gag gcC ccC cgC gaC 61. gtg aat gca aac act aaa cag gcc acc act gaG gag ccC cgc atc caG tta ccC act gtg 121. gat got ttc ogC ogC gca att ooC gca cac tgC ttc gaG agG gaC ctc gtG aaa tca atc 181. agG tat Ctg gtg caG gac ttt gcg gca ctG aca att ctG tac ttt gcC ctt ccC gcC ttt 241. gag tac ttt ggC Ctg ttt ggt tac Ctg gtG tgg aac att ttt atg ggC gtt ttt ggC ttc gog Ctg ttc gtc gtt gga cac gaC tgt ctt caC ggC tca ttc taC gat aat cag aat ctc aat gat ttc att gga cat atc gcc ttc ACC cca ctc ttc tct ccc tac ttc ccc tgg cag aaa agt cac aag ctG cac caC gcC ttc acc aac cac atC gac aaa gat cat gga cac gtg tgg atA cag gat aag gat tgg gaa gca atg co AGC tgg aaa aga tgg ttc aat co att 541. cor ttc tct ggC tgg ctG aaa tgg ttc cor gtg tac act CtG ttc ggt ttc tgC gat gga 601. too cac tto tgg coll tac too toa cto ttt gto coo aac tot gaa coo gtt cac tgt gta 661. atc tot gga atc tgC tgc tgt gtg tgC gca tat att gct cta aca att gct gga AGC tat 721. too aat tog tto tog tac tat tog oft coa ctt tot tto tto ogc ttg atg ctc otc att 781. gtt acc tat Ctg caC gtc gaC gtc gcc gcc gag gtg tac gag gct gat gaa tgg agc 841. tto gto cgG gga caG acc caG acc atc gat cgt tac tat ggC ctc ggC ttg gac aca acq 901. atg cac cat atc aca gac gga cac gtt gcc cac ttc ttc aac aaa atc cca cat tac 961. cat ctc atc gaa gca acc gaa ggt gtc aaa aag gtc ttg gag ccg ttg tcc gac acc caa 1021. tac ggg tac aaa tot caG gtg aac tac gat tto ttt gcc cgG tto ctg tgg tto aac tac aag ctc gac tat ctc gtt cac aag acc gcc gga atc atg caa ttc cga aca act ctc gag 1141. qag aag gca aag gcc aag tGa aag aat atc ccg tgc cgt tct aga gta caa caa cat 1201. ctg cgt ttt cac cgg ttt tgc tct aat tgc aat ttt tct ttg ttc tat ata tat ttt ttt 1261. got tit taa tit tat tot oto taa aaa act tot act tit oag tgo git gaa tgo ata aag 1321. cca taa ctc tt 1381.



Fx. 19



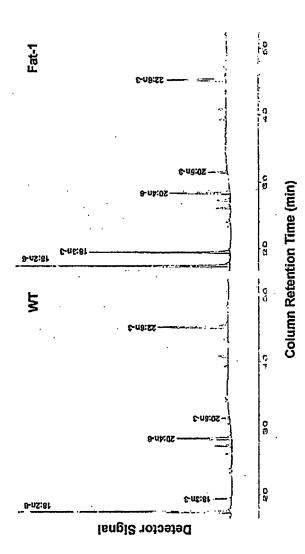
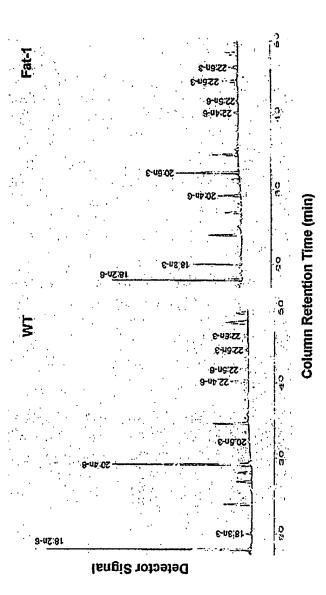


Fig. 21



This Page is Inserted by IFW Indexing and Scanning Operations and is not part of the Official Record

BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images include but are not limited to the items checked:

□ BLACK BORDERS
□ IMAGE CUT OFF AT TOP, BOTTOM OR SIDES
□ FADED TEXT OR DRAWING
□ BLURRED OR ILLEGIBLE TEXT OR DRAWING
□ SKEWED/SLANTED IMAGES
□ COLOR OR BLACK AND WHITE PHOTOGRAPHS
□ GRAY SCALE DOCUMENTS
□ LINES OR MARKS ON ORIGINAL DOCUMENT
□ REFERENCE(S) OR EXHIBIT(S) SUBMITTED ARE POOR QUALITY

IMAGES ARE BEST AVAILABLE COPY.

OTHER:

As rescanning these documents will not correct the image problems checked, please do not report these problems to the IFW Image Problem Mailbox.